

Figure 1. Overlap-extension-PCR fragment with purD deletion

Overlap-extension-PCR fragment with recA deletion

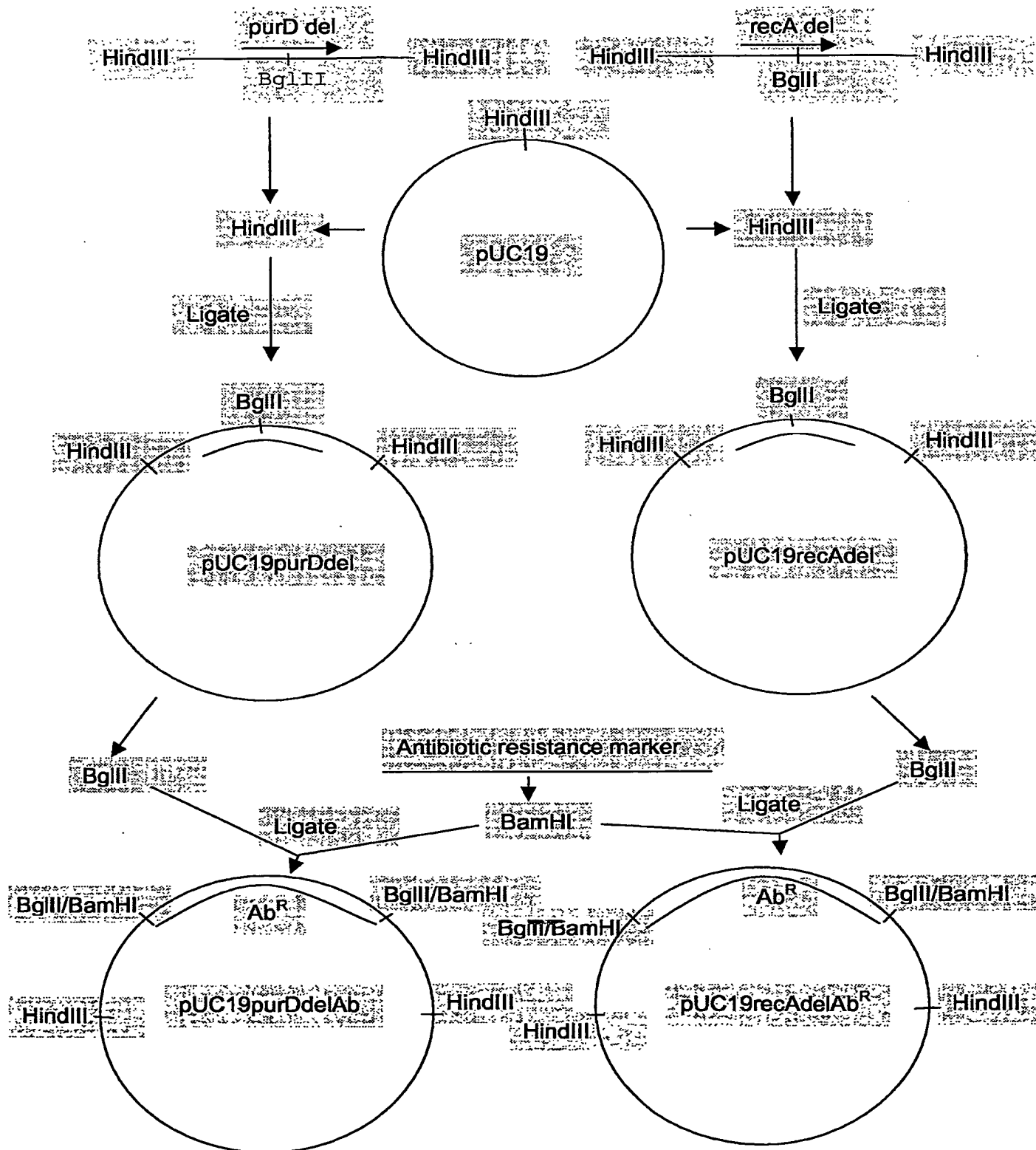


Figure 2A.

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1  GTTCGACCAA ACGGCTTGT GTGCGGTGAA ACATAGCACT CCTTGTTGGC TGGCTTTAGA TGATGATATT TTGCAAGCGT
   >>.....F5.....>>          CTTAAGCTTGA>>.....F13.....>>
                                   -----
                                   HindIII

81  ACCAAAAAGC ACACGACTGC GACCCGATTT CGATTTTGGG TGGCATTGTA ACTTTTAATA AAAAAGTAAC AAAAGCAGTG
161  GCAGAAAAAT GTAACGAGAT TTTCTTGAA ATCGTTGCTG CACCGAGCTT TGAGCCAGAG GCTTTGGAAG TTTTGTCTAA
241  AAAGAAAAAT TTGCGCGTGA TTGAAGTTAA AAATCCATTA AGCGATAAAA TGCAACTCGT GCAAGTAGAT GCGCGATTGC
321  TCGTGCAAGA AATCGACAAA TCGTTTAGCA ATGATTTTAA AGTAGTAACC GAAAAACAAC CTACCGAAAA GCAACTTTCT
401  GATTTGGAAT TTGCCATGAA AGTAGTAAA CATGTAAAGA GCAATGCCAT CGTGGTTGCC ACAAACGGAC AAGCTCTAGG
481  CGTGGGCACA GCGGAGACTA ATCGTATTG GGCAGCACAG CAGGCGATTG AGCGTGCAA GGAACAAACA CAAGAAAATC
561  TAGTTTTGGC TTCCGATGCC TTTTCCCAT TCAGAGATGT GGTAGATTAT GCAGCACAAG AAGGCATTAC AGCCTTGATT
641  CACCCAGGAG GAAGCATGCG CGACCAAGAG AGCATAGACG CGGCTAATGA ACACGGAATC CCGATGATCA TCAGCGGTAT
721  GAGACATTC TTACATTAA TCAAAAAATC TAAACAATA TTATCAATA TTCTAAAACA CAATAAGTAT GAATGCAAT
   >>...purD...>

801  GATTACAAA AAATACTCAT CGTAGGAAAC GCGCAAGAG AACACGCCAT CGGTTGAAA ATTAACAAG ACCACCTTC
   >.....purD.....>

881  TTGCGAGCTT TTCTTTCGC CAGGAAACGC TGAACCGAA CAAATTGGAA AAAACATCGT AGCTGAATCT AATTATGGCT
   >.....purD.....>
   <<.....OE-R.....<<AGATCTGGCGCTACGCTAGAAG
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                                   BglII

961  TAATGCTTTT TGCTCAACAA AATGATATAG ACTTAACGAT TGTAGGTCCA GAAGCAGAAT TGGTAGAAGG TATTGTAGAC
   >.....purD.....>

1041  TTGTTTGAAT CCAATCAATT AAGAATTTT GGTCCAGATA AGCGTGCGG TAAATTGGAA GGCAGCAAGG CTTTGTCCAA
   >.....purD.....>

1121  AGATTTTATG GAGAAATACG GCGTGCGCAC GGCTTTTGGC AAAAGTTTCA ACAATTTGT AGACGCTAGA GATTATGTAA
   >.....purD.....>

1201  AAGAGCTCAC GCAATTCCT ATCGTGATCA AAGCCAGTGG CTGGCAGCA GAAAAGGTG TGATCATCGT GCACNTACAA
   >.....purD.....>

1281  CTTGAAGCCG AAATACTTT GCGCAAAATC ATGGAAGACA AAACCTTGG CGAAGCAGG AACGAGGTCG TAATCGAGGA
   >.....purD.....>

1361  ATACTTAAAA GGTGTGGAAG TTTCTGTGCT TTCTATCTT AACATAAAG AAATTAAAC TTTCTGCCT GTAAAGACC
   >.....purD.....>

1441  ACAAGAAAAT CGGAAAAGGC GAAACAGGAC TCAACACGG CGGAATGGG GTAGTGGCTC CTAACCCGCA TTTTACCGAT
   >.....purD.....>

1521  GAGCAGTGA AGGAGTTTGA GAAAAACAT TTGCTCCCAA CAAAAAAGG GCTCTTGGCA GAAAAATGC ATTTTGCAGG
   >.....purD.....>

1601  CATTATTTTC TTTGGGCTTA TGATTACCGA GCATGGTATT TATCTATTG AATACAACAT GCGATTGGC GACCCAGAAA
   >.....purD.....>

1681  CCGAAGCACT TTGCCTTTG ATGGAGAATG ATTTAGTAGC CCTCATCGAT TCCGCAATAC ACCAGCAAGA CATTGAACCT
   >.....purD.....>

1761  AAATGGAAAA ACGAACATGC TTGCTGTGTA GTAATGGCG GCGGTGGCTA CCCAGGCACT TACGAAACTG GTTTTGAAAT
   >.....purD.....>

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1841 CCGAGGATTG AACAAAGTTG ATGTTCCCGT ATTTATTGCA GGAGCCAGAG AAGAAAGTGG AAAAATCTAC ACCACAGGCG
>.....purD.....>

1921 GGCGCGTGCT CAATGTGGTG GGAAGTGGCG CTACGCTAGA AGAAGCCAGA AAAGTGGCTT ACGAAAATAT CCATAAAATC
>.....purD.....>
GAGATCTGG>>.....OE-F.....>>

BglIII

2001 AATTTTGATT ATGAATATTA TCGCGAAGAC ATCGGGAAGA TATAATCTCG CTGATTTTAA ACCAAAACAT ATTTAAAAAC
>.....purD.....>>

2081 GCTTTTGTTA CTTTATAAAA CAAAGGCGTT TTTCTATTTT TGTGCCACTA TAACATGATT TAACCCATGA AAAAAACT

2161 AAAAATACTC ATTTTCTAC TGCTCATTCC TTGGGTTTAT GCCCTGATTT TAATCTTTAT AAATCCACCT ATCACCATTA

2241 CACAGCTGAG CAATTTATCT TATGTTTCT CCAGAACACA GCTCGCTTAT GATGAAATC CGGCTAGTGC TAAATGGGCT

2321 GTAATTGCAG CAGAAGACCA GAATTTTGCC ATTCATAATG GCTTTGATTT TAAAGAAATT AAAACCGCCT ACGAGAAAAA

2401 CAAAGCGGGC AAGAAATTGC GTGGCGGGAG CACCCTTTTCG CAACAACTG CCAAAATGT ATTTTGTGG CAAGGGCGCA

2481 CTTGGATTAG AAAAGGATTG GAAACCTACT GCACCTTTAT CATCGAAACG CTGTGGAGCA AGGAGCGTAT TTTGCAAGTT

2561 TACCTCAACA ATGCCGAAAT GGGCAAAGGC GTTTATGGCA TAGAGGCAGC GGCGCAATAT TATTTAAGA AAAACGCCTC

2641 ACAGCTCAGC CCTACCGAGA CGGCACGCAT CATTGCCTGC CTGCCAATC CCAAAAATA CAATNTAAC CCGCCAAGTG

2721 CCTACATCTC AAAACGCGGA CAATGGATTC TGCGCCAAGT GCGAACTTG AAAGGCGATA GGGCTCTGAG CGAGATTGTG

2801 AACACGCCCT AACGCCTGCC TCAACTCTTT GCACACAGTT TACCAACTCT CTGCGAAGAG TTCACAACT CTTCGCACAC

2881 ACTTCCCCAA GTCTTTGCAA AGAGTTGGGA GATACTTAGG CACAAAAAA AGGAACCTCA TGAATAGAGG TTCCCTCTTC

2961 CTAAAAGGA ATAAATAATA ATGTTTTTTA AGCTTTAGGC TTGGCTACTT TTTCAAAGCC TGCTGCCTTC ATGCTATCTA

HindIII

3041 GGATACGCTT GCCTGGGCGG TAGTTTACGC CTACCTTTTT GATTAAGCC GAATGAAAT CTTTCTCTGT ATCTGCCGCT
<<.....R8.....<

3121 CCACTGCTTA AAGTGGCATA GAGCGAGCCA AGCTTATCTA AACGAACGAT TTGCCCCGCT GCCAAGGCGT CTGAATTAC
<R8.<<AAGCTTAAG

HindIII

3201 ATTCTCTAGC GCAATGATAA CGCCACGAAT ATCTGCCTCG CTGAGTGCCG AAAACTTCTC GATTTGCTTA ACGAGCTGGT

3281 CTATATCCAT TTCTCCATCG CTTGCCACCA CGGCATAGTA TTTTGTGGC TCCCCTGGCT TGCTTGGGTT TCTACGCTGA

3361 ATTACATTGT ATTTTATGCT CATAATTACT CTATTTTAA TAGCCTCCCG ATGGATATAA AGTTACGCTA CAATTAGGGT

3441 CTCCATAAGC AAATCTATAC CCCTCTCTTT CATATTCCTT TCTCATTCTT CTGCTCCAT CTCTCAAGGC ATCCGCTCTA

3521 TTAAGTGTAT ACCCTCCTG AAGAAATGTG TCTGCACTTG AAGAAGATA TGAAGAGCTA TGAGAATCGT GCAACATAGT

3601 CCAAGCTCCA TCTTGAGCTA TAACATTGCT ATGACATGTA ACACCTATAG TATAATAAAA TCTCCTAGGA GGTGTGTTC

3681 CACCACCACC TCCAGAGCTA CTACTTTTTT TACATTGTCC ATTTTGGTTA GCATGATTTT GTCCGCCATC ACTTACTAAC

3761 TTCTTAGCTT CTGCTAAGGC TTTTCTCTT GCTTCTTTT CAGCATCTGC TTGGCTAATT CCACTCACTG CTGTAGCTGT

3841 CGCTTCTTTT TTATAGTTTA CCGAGGTTC ATAATAGCCA CTACTACAAT TGTTCTTGT AAAGTTTTTA TAAAAGATT

3921 GAGTTTGTGT TGAGGTGTAC CCTCCGAAAC CTTTACTTC TACAGTAAAG GTAGAACTCC CCATGCTTAC GGGGAAGGTG

4001 GCGATAGTAT ACGATTGCCC TGCCGGCATT TGTTTACTT GATACACTCC ATCTCCTCCC ACTTCTATGC TTGCCGTAA

4081 ATTACCACTA CCGCTAAAAG AGCCTTCTGC TATTTTGTAGT GTTAAATCAT TTATATCCCC TCCTTGTCTT TTGCAGAAG
4161 CTTTGTGTTAC ACTTACAGCA TCATAAGCTC CTTTCCATT GGTATAAGGT ATTTATATGG CCAAAC

Figure 2B.

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1  TAAAGCTGTA AWTGCTATA AACGCCCTTT AGGATAAAAT CTGCCATTTT TTGCAGTATT TTWATAGCTA AAATTTAGAA
   >>.....FrecAOR1.....>>

81  AACACCATCT CGAGTAAAGG AGCGTGTAGT GCTCGCCATC GTTGAGCGAT TGCCCACCCT CAATTGATTT GGGCGAATAC
   CTTAAGCTT>>.....F6.....>>
   -----
   HindIII

161  TTGAATAAAA TGGCATCTTC TAGCGACACA TTTGCGCAG AAATCATGCA AAAAGCCCCG CATAAAAAGC TGAATAAAAA
241  WGCTAWTYTT CTGTTTTAAA AAAACTCATA AATCCCCCA AATATAGAAA TATTCTGTGA AAAGTGCAG TTTATTAACA
   <<....<

321  CTATGTGCTT GCTTTTAATG AAAAAAGTAG ATTATTTTTC CGAATCCGAA AGTTTATTTA CGCCCCATCC GATGCCTAGT
   <..FrecA-4...<<

401  CCCMSCGATA GCCATGATTA ATACAAATAC AATTAAATCA WATTTTTCMC MTWWACCATA GCACAACACT TGCTAGCTCA
481  ACGAGTACTA GAGTGGTAAA AAGGATTTTT TGACGATTAT TCATGATTTT ATTTTCTCA AAGGTAAATA TTTTAAACCA
561  TAATTTTACA AATCTTAAA TCTATTTAAA TAATAGAGAA ACCAGAAAAA AATCGTATTT TTACGGAATG AATAAAATGT
641  TACAAGTAGG CGATAAAATG CCCGATTTC AAGGTGTAGA CCAATTGGG AAGGAGCATT CATCTGCCGA TTTCAAAAAT
721  CAGAAATTAG TCGTTTTTTT CTACCCAAA GCCAGTACGC CAGGTGCAC GGCAGAGGCT TGCAACATCA ACGATAATCT
801  TGATGCGCTA AAAGCACAG GCTACCAAGT GATAGCGGTG AGTGCAGATT CGGTAGAAAA ACAACGAAAA TTCAGTGATA
881  AATACGATTT TAAATCCCT GTGATTGCCG ATGTGGATAA GAAAATTATT GAAGCATTG GCGTGTGGG CGAAAAGAAA
961  TTCATGGGTA AAACCTATGA CGGAATTCAT CGTACGACAT TCATTATTGA TGAAACGGA GTGGTGGAGC GCGTGATAGA
   >>.....F7.....>>
   -----
   EcoRI

1041  AAAAGTGAAA ACAAAGATC ATACCAATCA AATTTTAAAT TCAGAAAAAT AAAAATATGA GCGAAATAGA CGAAGCGAAA
   >>.....recA.....>

1121  AGGAAAGCAC TCCAGCTAGT GCTTGATAAA ATGGACAAA GCTATGGTAA AGGTGCCGTG ATGATGATGG GCGACAAAGC
   >.....recA.....>
   <<.....OER1.....<

1201  CATAGACGAA AATATTCCAG TAATCCCTAC GGGGTCTCTA GGTTTAGATT TAGCCTTGGG CGTGGGAGGG TATCCGCGCG
   >.....recA.....>
   <CGAGATCTCGTGCCTGCGGT
   -----
   BglII

1281  GTAGAATCGT GGAGATTTAC GGTCCAGAAT CTTCTGGTAA AACCACCTTG GCAATTCATG CCATTGCCGA AGCTCAAAG
   >.....recA.....>

1361  TCTGGCGGAA TTGCAGCTTT CATCGATGCA GAGCACGCAT TTGATAGATA TTACGCAGAA AAATTAGCG TAGATGTTGA
   >.....>

1441  GCATTTAATT ATCTCTCAGC CAGATAATGG GGAGCAAGCT TTAGAAATTG CCGATAACTT AATCCGTTC GGTGCAATTG
   >.....recA.....>
   -----
   HindIII

1521  ATATTATTGT AATCGATTG GTAGCGGCTT TAACGCCAAA GTCGGAAATC GACGGAGATA TGGCGGATTC CAAAATGGGA
   >.....recA.....>

1601  TTGCAAGCGC GTTGATGTC TCAAGCCTTG AGAAAGCTCA CGGGAATAT CAATAAAACC AAATGTACTG CTATTTTCAT
   >.....recA.....>

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1681 CAACCAATTG AGAGAGAAAA TCGGTGTGAT GTTCGGTAGT CCAGAAACCA CAACGGGTGG TAATGCACTT AAATTCTATG
>.....recA.....>

1761 CATCGGTGCG TCTAGACATT CGTCGTTCTA CTCAGATTAA AGATGGGAAC GATGTCATCG GAACTTGAC TCGCGTAAAA
>.....recA.....>

1841 GTAGTGAAAA ACAAAGTAGC TCCGCCATTC CGTAGTGCAG AATTCGACAT TATGTATGGC GAAGGAATCT CTAAGCAGG
>.....recA.....>

EcoRI

1921 CGAGATTTTA GACATTGCTA CCGATTAGA AATCGTGAAA AAAAGTGGCT CTTGGTATTC TTATGCAGAT ACTAACTAG
>.....recA.....>

2001 GACAAGGGCG AGATGCCGTG CGTGCGGTAT TGAAAGATAA TCCAGAATTA GCCGAAGAAT TAGAAGAGAA AATTAAAGAA
>.....recA.....>
CGAGATCT>>.....OEF1.....>>

BglIII

2081 GAATTAGAGA AAAAATAGAT TTTTAGTTT TTTTAATTAA ACGAAAAATC CGTTCACTTT GTTGAACGGA TTTTATTATG
>.....recA.....>>

2161 CTTGAATGAA TTTATTTCCA ATGGATTGAA TAGCCATGCA CTTTAAATC TTCGCTATCA TAAGTGATT TTTGTGCGT

2241 GTTGGGATAG CAAACTTTAA GTCCTGCGTA TTTGGCAATG GCATGTCCTG CGGCAATGTC CCAAAAGTTT ACAGGTCTAA

2321 AGCGGGTGTA CTCCGTAGCC CACCGATCGG CAATTAGCCC AAGTTTGATA ACGCTTCCCA TAGGCTTTGT GCGGAAAAAT

2401 TCGATGTCGG ATTTAATTTT TTTGATGTAT TCCTCGGTGC CAGGATCCAT GTGGAATTTG CTACAAAGAA AAGTGTAATC

2481 TTCGGGCAAA TCCATGGTAG GAATTGGCTT GCTGTGTTTC ATCAATTGTT CAAAAAATC CGATTTCAGA GCCATTTTGT

2561 GCAATTGTTG TTGAGTCCCG ATGAATTAC GAGAAGGGCA TTTATCGCTA CCGAAATAGA ACAATCCAAG CGATGGGGCG

2641 TACAAAATC CTAGCTTAGC CGTATTATTC TCAACTAAGC CTAGACACAC GCAATATTCA TCTGTTTTGT TGACAAAATC

2721 CATGGTGCCA TCAATAGGT CTGCAATCCA ATAGGTGGGC GTATTTCTAA TTTCTGTAA AGAATCCTTA TCTCCTTCTT

2801 CACTAAAGTA TGGAAATGTCT GTAAAGGAAA CATGTTTTTG CAAGATTTTG TTGGCGGCTA AATCTGCACT TGTAACAGGC

2881 GATCCGTCGG CTTTGGTCTC GGTGGAGAAT CCGTTTTGGA TTGTTTTAAA ACCTCTTCGC CAGCAAGTGC TACAGCCCGT

2961 GTTGCATT TTAATAAATT CATAATCATT CTTTATTCT CGAACAAAGT CAAATAATTC TCTGTATTAA AAAATAATTT

3041 TGGCGATAAA AATTAAAATT TATATATAAA ATATCTCTGC AAAAAACCAA ATCAAATATT TAGTGAAATA AAAAAATTA

3121 GATTGTAAAT TTGCCTTATG TTTTATGAGA ATACCATAAA TCATAGAAAA AATACGGGCT GGATCGAAGT AATCTGTGGC

3201 TCTATGTTTT CGGGCAAAAC CGAAGAGTTG ATTCGTAGAG TGAACGAGC CGAATTGGCT GGGCAAAAGG TAGAAATCTT
<<.....R5.....<<AAGCTTAAG

HindIII

3281 TAAACCCGCA ATTGATAAAC GCTACGATGA GCAAGATGTG GTATCGCATG ATGAAAACAA AAAACAAGCA ACCCCGATTG

3361 AGGCGAGTTC TAACCTGCCC ATTTTAGCAA GCGATTGTGA TGTGGTGGGG ATAGATGAGG CTCAATTCTT TGACGAAGGA

3441 ATTGTTGAGG TGGCAATCT TTTAGCTAAT TCGGGGAAAA GAATAATTAT TCGGGGATTA GACATGGATT TTAAAGGTCG
<<.....RrecAOR1.....<<

3521 TCCATTTGGT CCTATGCCAA ATTTAATGGC GGTAGCGGAA TATGTGACCA AAGTGCATGC AATCTGTGTG AAAACAGGGA

table 5

group	no. of chickens	Treatment			Results	
		vaccination at day 1	challenge at day 25	challenge at day 31	% of max air sac score at day 10 (safety)	% of max air sac score at day 38 (efficacy)
1	25	NDV	NDV	WT-OR aerosol	2.5	25 ^b
2	25	NDV	NDV	WT-OR aerosol	7.5	23 ^b
3	25	NDV	NDV	WT-OR aerosol	68	10 ^b
4	25	NDV	NDV	WT-OR aerosol	0	47
5	25	NDV	NDV	NDV	0	2

^b Significantly different ($p < 0.05$) compared to the controls (group 11) using two-sided Mann-Whitney U test

table 6

group	no. of chickens	Treatment			Results
		vaccination at day 1	day 30	challenge day 35	
1	15	PurD aerosol	NDV	WT-OR aerosol	no reduction
2	15	NDV PurD aerosol	NDV	WT-OR aerosol	54% ^b
3	15	NDV	NDV	WT-OR aerosol	no reduction
4	15	MAS	NDV	WT-OR aerosol	no reduction
5	15	MAS PurD aerosol	NDV	VT-OR aerosol	50% ^b

^b Significantly different ($p < 0.05$) compared to the controls (group 1) using two-sided Mann-Whitney U test